POSTFERTILIZATION DEVELOPMENT AND THE NATURE OF THE CONNECTING CELL IN *AGLAOTHAMNION HALLIAE* (CALLITHAMNIEAE, CERAMIACEAE)

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ABSTRACT — An investigation of early postfertilization stages in *Aglaothamnion halliae* (Collins) Aponte, Ballantine *et* J.N. Norris from North Carolina yielded results that were at variance with previously published observations for the tribe Callithamnieae.The earliest events were identical with previously published reports: the fertilized carpogonium divides longitudinally into two cells, the supporting cell and an opposite periaxial cell each cut off auxiliary cells, and each of the two carpogonial derivative cells cuts off a connecting cell. Following fusion of a connecting cell with an auxiliary cell, additional stages were observed: the diploid nucleus divides and one of the daughter nuclei enters the auxiliary cell, while the other is extruded and cut off in an external cell that resembles the original connecting cell; the auxiliary cell cleaves transversely by an incomplete septum into a foot cell containing one or two haploid nuclei and a gonimoblast initial containing the diploid nucleus. Gonimoblasts develop in the usual manner. The additional postfertilization stages seen here in *Aglaothamnion* have not been reported previously in the Callithamnieae or elsewhere in the Ceramiaceae.

RÉSUMÉ — Une étude des événements suivant la fécondation, chez *Aglaothamnion halliae* (Collins) Aponte, Ballantine *et* J.N. Norris de Caroline du nord, a conduit à des résultats qui varient par rapport aux observations publiées jusqu'à présent pour la tribu des Callithamniae. Les premiers événements sont identiques aux observations publiées antérieurement : le carpogone fécondé se divise longitudinalement en deux cellules, la cellule support et une cellule périaxiale opposée, chacune produisant une cellule auxiliaire, puis, chacune des deux cellules dérivant de la division du carpogone produit une cellule de jonction. Après la fusion d'une cellule de jonction avec une cellule auxiliaire, des événements additionels ont été observées : le noyau diploïde se divise et un des noyaux fils pénètre dans la cellule auxiliaire, tandis que l'autre est expulsé et isolé vers l'extérieur, dans une cellule qui ressemble à la cellule de jonction ; la cellule auxiliaire se divise transversalement au moyen d'une cloison incomplète en une cellule pied contenant un ou deux noyaux haploïdes et une initiale du gonimoblaste contenant le noyau diploïde. Le gonimoblaste se développe de la manière habituelle. Les événements additionels suivant la fécondation observés ici chez *Aglaothamnion* n'ont pas été rapportés auparavant chez les Callithamniae ni chez aucun autre groupe au sein des Ceramiaceae. (Traduit par la Rédaction)

KEY WORDS: Algae, *Aglaothamnion*, auxiliary cells, *Callithamnion*, Callithamnieae, Ceramiaceae, Ceramiales, connecting cells, Rhodophyta.

INTRODUCTION

Oltmanns (1904) established the Ceramiales to include taxa in which an auxiliary cell is cut off from the supporting cell of the carpogonial branch and sometimes also from adjacent periaxial cells in the fertile whorl after fertilization. In a few instances it has been shown that the supporting cell enlarges after fertilization and functions directly as the recipient of the connecting cell, dividing only afterwards (Dixon, 1964; Baldock, 1976). Except for this minor modification, Oltmanns' key character has proved to be diagnostic for all families and genera placed in the Ceramiales. Much less is known about mechanisms by which a derivative of the fertilization nucleus is transferred from the carpogonium to an auxiliary cell. In no instance has it been shown that transfer takes place directly without the prior division of the zygote nucleus and, indeed, most evidence indicates that the fertilization nucleus normally divides twice to produce either three or four nuclei before transfer occurs (Hommersand & Fredericq, 1990). Nuclear transfer has been reported to take place by direct fusion of the carpogonium with the auxiliary cell, by a connecting tube linking the carpogonium to the auxiliary cell, or indirectly by means of a connecting cell or a short two- to three-celled connecting filament (Dixon, 1973).

In 1898, Oltmanns investigated the formation of auxiliary cells and followed the transfer of the sporogenous nuclei (diploid nuclei) by means of sporogenous cells (= connecting cells) in *Callithamnion corymbosum* (J.E. Smith) Lyngbye using material stained with Heidenhain's iron alum hematoxylin. Based on earlier studies and his own observations, Oltmanns (1904, 1922) expressed the opinion that "*Callithamnion*, the most studied example, may serve as the representative type for the Ceramiaceae". Connecting cells have been illustrated occasionally for members of the Callithamnieae since Oltmanns; for example, by Kylin (1923) for *Aglaothamnion byssoides* Arnott *ex* Harvey *in* W.J. Hooker) L'Hardy-Halos (as *Callithamnion furcellariae* J. Agardh); by G. Feldmann (1941) for *Seirospora giraudyi* (Kützing) De Toni, and by O'Kelly & Baca (1984), who followed the time course of carpogonial branch and carposporophyte development in an undescribed species of *Aglaothamnion* (as *Callithamnion cordatum* Børgesen, see Rueness & L'Hardy-Halos, 1991). More often, connecting cells have been illustrated in proximity to auxiliary cells that have already produced gonimoblasts, as if they persisted in the position in which they were initially formed. Examples include: Itono (1977) in *Callithamnion aglaothamnioides* Itono, and Kajimura (1990) in *Callithamnion callophyllidicola* Yamada.

Connecting cells are fairly large and filled with cytoplasm in some Ceramiaceae, whereas in others they are small and relatively free of cytoplasm. The larger ones, which were referred to by Wollaston (1968) as "tube-like connecting cells" in *Heterothamnion muelleri* (Sonder) J. Agardh may well have been a tube that has detached from the carpogonial end after uniting with the auxiliary cell. The Dasyaceae have long been thought to possess connecting cells (Oltmanns, 1898; Parsons, 1975), whereas the Delesseriaceae have been said to lack them, except in *Caloglossa* (Papenfuss, 1961). The claim by Maggs & Hommersand (1993) that connecting cells are widely distributed in the Delesseriaceae was illustrated for the first time in *Myriogramme* by Hommersand & Fredericq (1997). Diploidization has been said to be mediated by connecting cells in the important case of *Polysiphonia* (Hommersand & Fredericq, 1990) and by direct fusion between the carpogonium and the auxiliary cell (Broadwater & Scott, 1982).

In view of the wide disparity among published observations and opinions as to just how a derivative of the fertilization nucleus reaches the auxiliary cell and what happens immediately afterward, it is appropriate to reinvestigate the diploidization of the auxiliary cell in a representative of the Callithamnieae. It would have been preferable to study this process in the type species of *Callithamnion*, *C. corymbosum*, investigated previously by Oltmanns; however, in the absence of suitable material of that species the present study was carried out on *Aglaothamnion halliae* from North Carolina.

Aglaothamnion halliae (Collins) Aponte, Ballantine *et* J.N. Norris is a new combination for a plant previously described as *Aglaothamnion westbrookiae* Rueness *et* L'Hardy-Halos and prior to that known as *Callithamnion byssoides* Arnott *ex* Harvey *in* W.J. Hooker, or *C. pseudobyssoides* P. Crouan *et* H. Crouan. (See Schneider & Searles, 1991; Rueness & L'Hardy-Halos, 1991; and Aponte *et al.*, 1997; for the complete synonymy for this species and records of its North American distribution). *Aglaothamnion* is separated from *Callithamnion* primarily on the basis that vegetative cells are mostly uninucleate in the former and mostly multinucleate in the latter (Feldmann-Mazoyer, 1941; Maggs & Hommersand, 1993). The reproductive development is essentiallly the same in both genera.

MATERIALS AND METHODS

Morphological studies of *Aglaothamnion halliae* were made on material found growing attached to the seawall connecting to the jetty on the north side of Masonboro Inlet, Wrightsville Beach, New Hanover Co., North Carolina, by M. H. Hommersand on May 13, 1980, and fixed in 8-10% Formalin/seawater. Wholemount preparations were stained with Wittmann's aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1965) and transferred to Piccolyte® as described in Hommersand and Fredericq (1997).

OBSERVATIONS

Stages of procarp development and spermatial attachment to the trichogyne were not followed in this study. Figure 1 illustrates an early postfertilization stage in which the supporting cell of the carpogonial branch and the cell borne opposite it on the fertile axial cell have begun to enlarge and become densely filled with cytoplasm. The carpogonium lacks a trichogyne remnant and contains an enlarged, presumably diploid nucleus. The second and third cells of the carpogonial branch each contain two nuclei at this and subsequent stages. The carpogonium next cleaves longitudinally into two cells (Fig. 2). Remnant cytoplasmic strands are sometimes seen linking the two cells; however, typical pit connections containing pit plugs are not formed. By this time, the first cell of the carpogonial branch also usually contains two nuclei. Occasionally the carpogonium divides longitudinally twice, cutting off a derivative cell on each side (Fig. 5), but this is a comparatively rare event. Each of the two cells derived from the division of the carpogonium next cuts off a connecting cell adjacent to a prominent beak that develops on the auxiliary cell (Fig. 3). The connecting cell consists of a densely compact nucleus surroun-

ded by a hyaline region and an outer cell membrane or thin cell wall. Pit connections containing pit plugs were never formed in association with the connecting cells. The nuclei in the connecting cells apparently initiate mitosis, passing into metaphase prior to fusing with an auxiliary cell (Fig. 4, left arrow). A connecting cell fuses with the beak portion of the auxiliary cell and continues undergoing mitosis as illustrated by the anaphase stage shown in Figure 4 (right arrow). The mitotic stimulus extends to the division of the haploid nucleus below which is seen in anaphase (Fig. 4, hn). Once nuclear division is complete, one of the nuclei enlarges inside the beak portion of the auxiliary cell (Fig. 5, arrowheads) while the second smaller nucleus is extruded to the outside on the side on which the connecting cell originated (Fig. 5, arrows). This latter cell persists through later stages of gonimoblast development and is easily confused with the connecting cell. The two haploid nuclei at the base of the auxiliary cell become separated into a foot cell by an incomplete septum without the formation of a pit plug (Fig. 5). The nucleus in the beak portion of the auxilary cell next divides (Fig. 6). One of the daughter nuclei moves into the central portion of the auxiliary cell while the other remains in the beak. In later stages the main portion of the auxiliary cell forms the terminal gonimolobe while the beak portion is cut off as a one-celled lateral gonimolobe initial (Fig. 7, arrowheads). This cell will ultimately produce the first lateral gonimolobe. Each of the cells derived from the first division of the nucleus inside the auxiliary cell that were disposed to the outside remain clearly visible, and resemble the original connecting cells (Fig. 7, arrows). Likewise, the supporting cell, foot cell, primary gonimoblast cell and cells of the gonimoblasts and gonimolobes, as well as the four cells of the carpogonial branch and derivative cells of the carpogonium persist and are easily recognized during early stages of gonimoblast development (Fig. 7). Subsequent stages of gonimoblast development and carpospore maturation take place as reported by O'Kelly & Baca (1984).

DISCUSSION

Most of the postfertilization stages described here for *Aglaothamnion halliae* are consistent with those reported by Oltmanns (1898, Pl. VI, figs. 1-13) for *Callithamnion corymbosum* and reviewed by him (1904, 1922). Figures 3 & 4 in Oltmanns (1898) correspond to Figs. 1 & 2 here. His figure 5 shows a disrupted procarp with 1- and 2-celled connecting cells containing nuclei in which the cells are linked by pit connections. Since pit plugs were absent in my material, their possible presence in *Callithamnion corymbosum* requires reinvestigation. Unlike some later workers, Oltmanns used the term "sporogenous cell" (= connecting cell) only for the evanescent cell that transfers the "sporogenous nucleus" to the auxiliary cell (Oltmanns, 1898, pl. VI, fig. 6). Similar connecting cells are shown here in Figure 3. Figures 7-10 in Oltmanns show the division of the sporogenous nucleus and the migration of one of the nuclei into the beak of the auxiliary cell followed by the division of the auxiliary cell into a foot cell and a "central cell" (= gonimoblast initial). The foot cell contains the undivided auxiliary cell nucleus and the unmodified sporogenous nucleus. His observation that the auxiliary cell divides into a foot cell containing a haploid and a diploid nucleus and a primary gonimoblast cell containing a diploid nucleus in *C. corymbosum* was reaffirmed by Oltmanns in a color diagram in 1904 (Fig. 449a, 7-9) and again in 1922 (Fig. 591, 7-9). This pattern has been reported elsewhere in *Seirospora orientalis* of the Callithamnieae (Kraft, 1988) and *Spyridia* of the Spyrideae

(Hommersand, 1963), and may prove to be widespread in the Ceramiaceae when nuclear events have been described in other tribes.

The observation reported here that one of the nuclei resulting from the first division inside the auxiliary cell is extruded and cut off to the outside in a persistent cell that resembles a connecting cell is unique, so far, for the tribe Callithamnieae and the Ceramiaceae. Interestingly, Oltmanns (1898, pl. VI, Fig. 12) illustrates two persistent extruded cells in the same position as the ones shown here in Figures 6 and 7. Since his fig. 12 is not one of the figures reproduced later in 1904 or 1922, the significance of this cell may have been overlooked. While the sporogenous nucleus present in each of these two cells is labeled, the cell itself is not. Clearly, Oltmanns never confused this cell with a connecting cell, but he did not clarify the relationship of these cells to the foot cell shown in fig. 13 which lacks a diploid nucleus but contains two haploid nuclei.

The incomplete septation of the auxiliary cell into a foot cell containing only the haploid nuclei and a gonimoblast initial that receives the diploid nucleus has also not received specific mention for the Ceramiaceae. Huisman & Kraft (1992) reported that the haploid nuclei are deleted from the auxiliary cell into a 'disposal' cell after diploidization in *Guiryella repens* Huisman *et* Kraft. In this species the disposal cell is cut off laterally from the auxiliary cell and persists as a detached cell. It differs in this respect from a 'foot' cell which occupies an intercalary position between the supporting cell and the primary gonimoblast cell. As these authors point out, the disposal cell performs a similar function to a foot cell in serving as a nuclear-segregating device which may be necessary for compartmentalizing the diploid carposporophyte from the haploid gametophyte generation.

Two types of foot cells have now been documented for members of the tribe Callithamnieae, one in which the foot cell contains both a diploid and one or two haploid nuclei, as in *Seirospora orientalis* (Kraft, 1988), and one in which the foot cell contains only haploid nuclei, as in *Aglaothamnion halliae* reported here. The situation in *Callithamnion corymbosum* is ambiguous and requires reinvestigation. Future studies will establish whether the two types are variable or genetically fixed within a species, and whether or not these postfertilization behavior patterns may serve as diagnostic characters at the species or genus level in the Callithamnieae.

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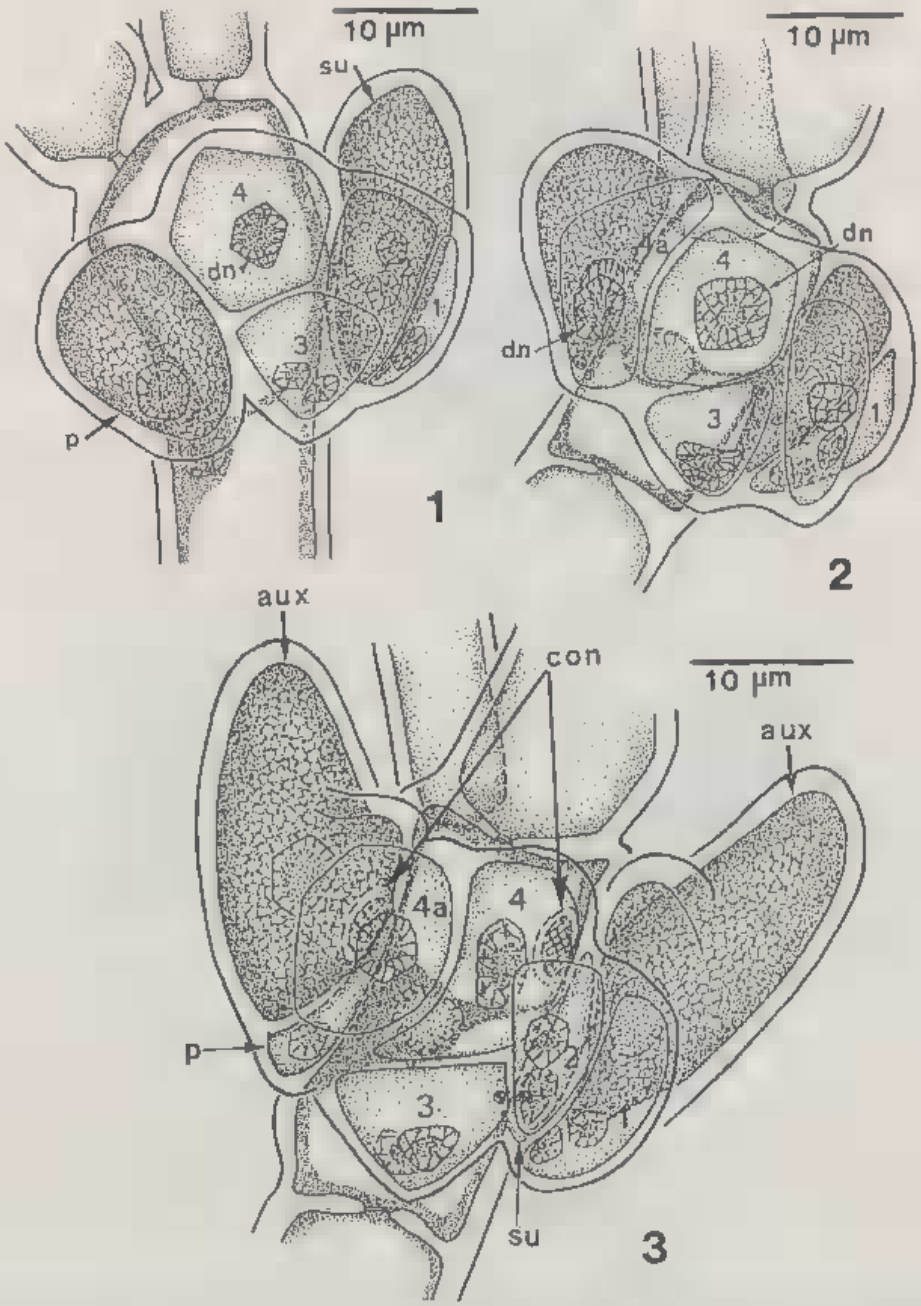
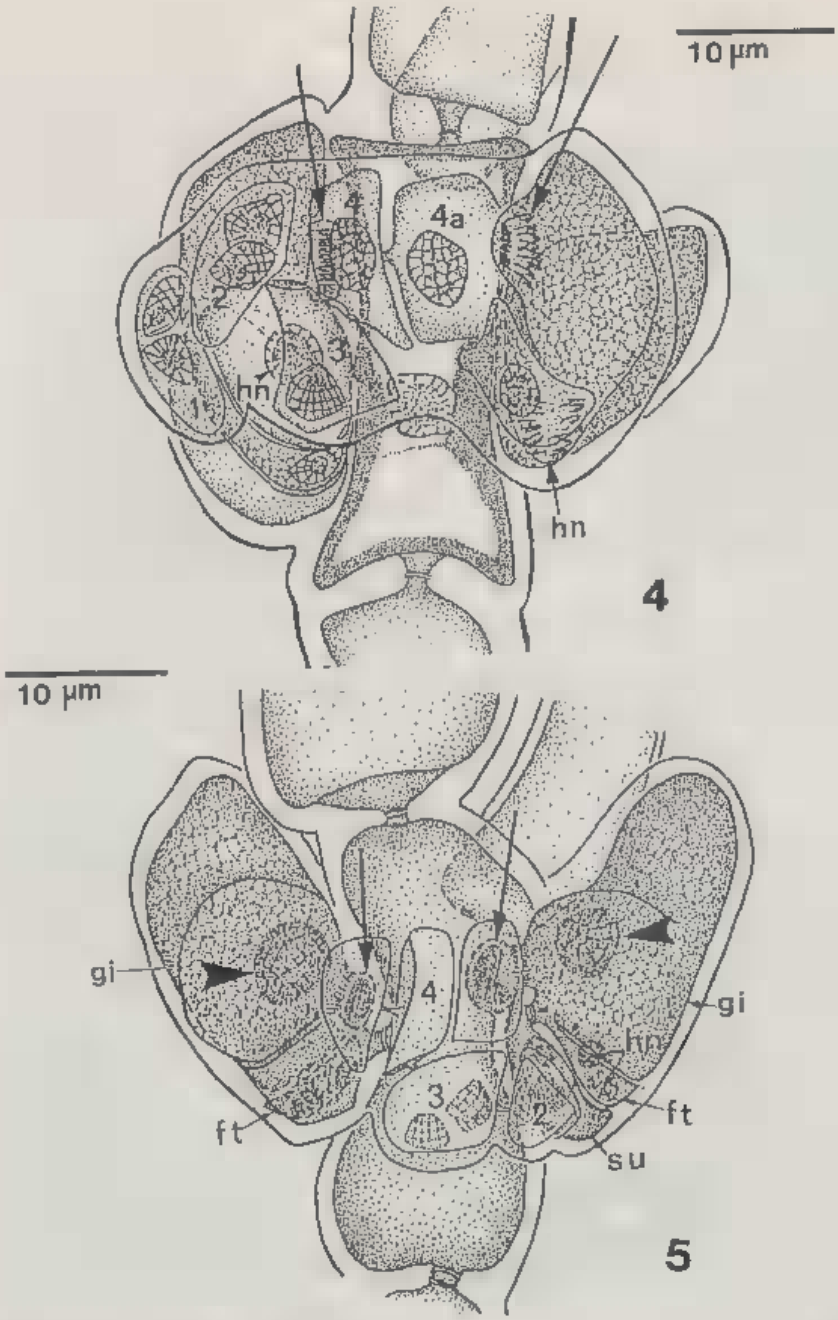
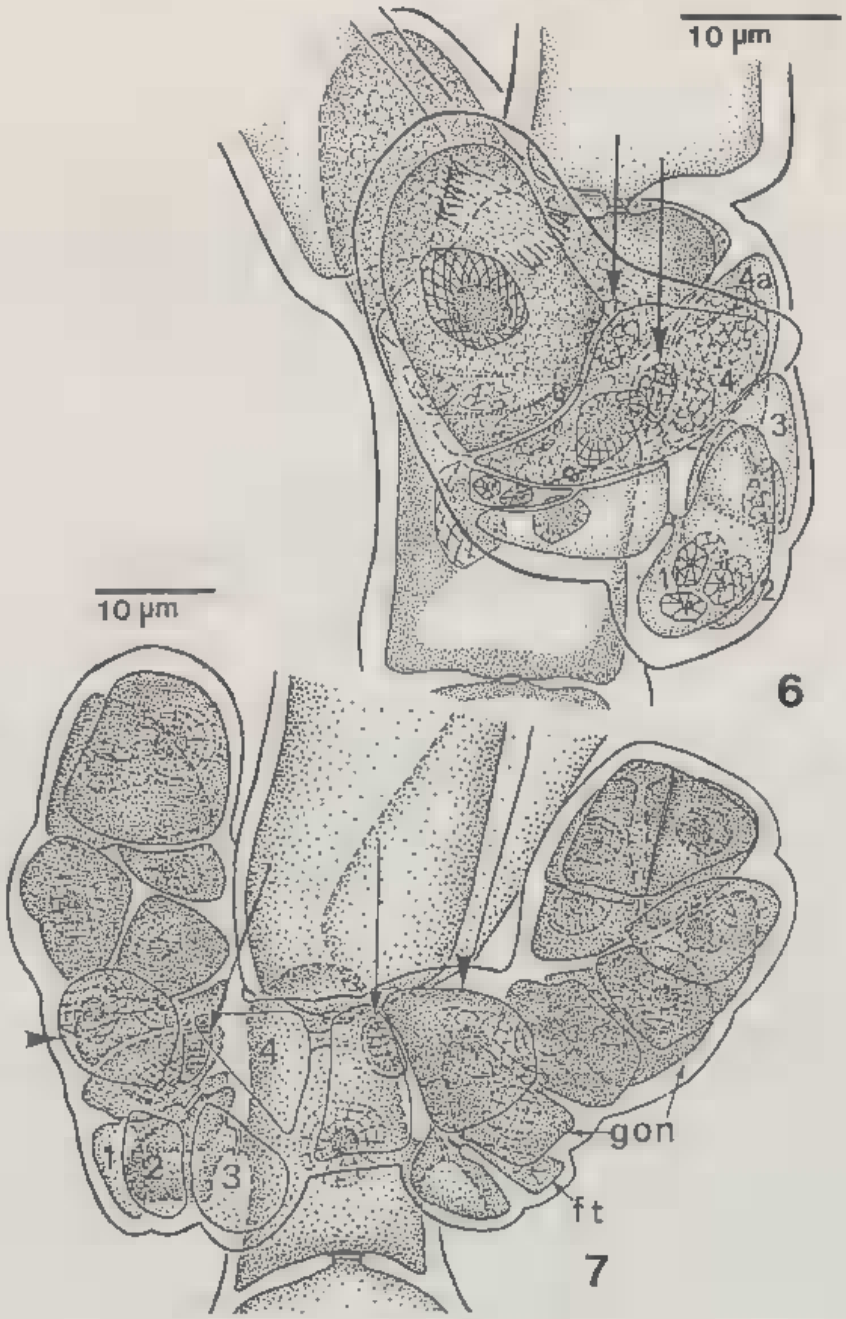


Figs 1-3. *Aglaothamnion halliae*. Fig. 1. Early post fertilization stage showing an enlarged supporting cell (su) bearing a four celled carpogonial branch (1-4) and an enlarged fertile pericentral cell (p) on the opposite side. The enlarged nucleus (dn) in the carpogonium is presumed to be diploid. Fig. 2. The carpogonium has divided longitudinally into two cells (4, 4a) containing diploid nuclei (dn). Fig. 3. The supporting cell (su) and fertile pericentral cell (p) have each cut off an auxiliary cell (aux) with a prominent lateral beak. Derivatives of the carpogonium (4, 4a) have each cut off connecting cells (con).



Figs 4-5. *Aglaothamnion halliae*. Fig. 4. The connecting cell cut off from the carpogonium (4) is in metaphase (arrow on left), and the one cut off from the carpogonial derivative cell (4a) has fused with the auxiliary cell and is in anaphase (arrow on right). The haploid auxiliary cell nucleus (hn) at the base of the auxiliary cell is also in anaphase. Fig. 5. Division of the nuclei inside the two auxiliary cells is now complete with one nucleus enlarging inside each auxiliary cell (arrowheads) and the other, extruded in the direction of the original connecting cell, is being pinched off (arrows). Auxiliary cells on each side (aux) have septated by an incomplete cleavage into a foot cell (ft) containing two haploid nuclei (hn) and a gonimoblast initial (gi).



Figs 6-7. *Aglaothamnion halliae*. Fig. 6. Transverse longitudinal view of developing gonimoblasts. The one on top is two-celled and the one behind is one-celled with the nucleus in late anaphase. The small cells containing the nuclei extruded after the first nuclear division are distinct (arrows). Fig. 7. Paired young gonimoblasts. A foot cell (ft) is seen at the base of the gonimoblasts (gon) on the right. The terminal portion has produced the first gonimolobe, while the lateral beak has been cut off as a one-celled gonimolobe initial (arrowheads). The cells containing the extruded diploid nuclei from the first division of the auxiliary cell have persisted (arrows).